Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Combined oxaloacetate and dehydroepiandrosterone treatment: a new neuroprotective startegy

János Fuzik

Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Stroke is accompanied by the development of neuronal and functional loss. Under ischemic brain pathological conditions interstitial glutamate (Glu) concentration increases to an excitotoxic level. Decreasing blood Glu concentration enhances the brain-to-blood efflux of Glu. The so called Glu scavenging from the brain moderates Glu excitotoxicity which contributes to the neuronal loss and long-lasting neurological deficits seen in stroke. Inflammatorical events take place in the affected area a few days after the excitotoxic period. In the aspect of therapeutic window Glu scavenging has to be done immediately after ischemic insult whereas antiinflammatory treatement is effective in the following 48 hours after cerebral ischemia.

4VO (four-vessel occlusion) as a global cerebral ischemic model was used to evaluate the neuroprotective effects of oxalocetate (OxAc) as a Glu scavenger and Dehydroepiandrosterone (DHEA) as antiinflammatic agent and the combined treatement.

ECoG recordings were carried out for the validation of the global ischemic intervention and for the detection of the effect of OxAc on post-ischemic ECoG pattern and on Burst-Supression ratio (BSR). Furthermore, power spectral density (PSD) and changes in ratio of frequency bands were measured. *In vitro* extracellular field-EPSP amplitudes were measured, LTP induction and I/O curves were recorded in the CA1 subfield of rat hippocampal brain slices.

FlouroJade C staining was used to visualize the degenerated CA1 neurons, Cresil-violet staining was used to estimate the thickness of the CA1 pyramid cell layer.

OxAc (20mg/100g bw) administered right after the ischemic insult decreased the formation rate of post-ischemic ECoG pattern. In the in vitro experiments both OxAc (20mg/100g bw) and DHEA (2mg/100g bw) resulted in a mild increase of the impaired synaptic plasticity in the CA1 region. The combined OxAc mediated glutamate scavenging and the DHEA treatement together were able to moderate the ischemic damage in the 4VO group and increased synaptic plasticity.

Supervisor: Tamás Farkas
E-mail: tfarkas@bio.u-szeged.hu

The biosynthetic pathway of PGE2 and its role in the virulence of *Candida parapsilosis*

Zsuzsanna Grózer

EMBO Candida Work Group, Department of Microbiology, University of Szeged, Szeged, Hungary

Candida parapsilosis is often the second most commonly isolated *Candida* species from blood cultures, and it even outranks *Candida albicans* in some European, Asian, and South American hospitals. *C. parapsilosis* is an opportunistic human pathogen, that can colonize and cause disease on immuno-compromised patients (with AIDS, organ transplantation ect.) or in particular patient groups such as neonates or elders

Despite the increasing clinical importance, little is known about the virulence factors of *C. parapsilosis*. Thus, in our recent study we investigated the biosynthetic pathway of prostaglandin E2 (PGE2), a putative virulence factor of *C. parapsilosis*. Prostaglandins are fatty acid metabolites build up of 20 carbon atoms. Mammalians produce immune response regulator prostaglandins from arachidonic acid by the contribution of *COX1* and *COX2* cyclooxigenases. Although fungi do not possess cyclooxigenase homologes, several pathogenic species are able to produce prostaglandins from host originated arachidonic acids. In case of *C. albicans* the fatty acid desaturase homolog ole2 and the multicopper oxidase homolog fet3 enzymes were identified as potential key factors of the prostaglandin biosynthetic process. Due to its ability to block Th1-type, and promote Th2-type immune response, fungal Prostaglandin E2 can move the host's immune response towards helping the fungi to colonize and to carry out chronic inflammation. In our recent study we investigated the role of the putative fatty acid desaturase CpOle2 in the prostaglandin biosynthesis of the emerging human pathogen *C. parapsilosis*. We generated a homozygous *OLE2* deletion mutant through repeated application of a caSAT1 flipper KO cassette. We characterized the pseudohypha production, FBS utilization ability, growth ability on different pH and temperature of the *OLE2* deletion mutant in comparison to that of the wild type strain and we found that mutant strain showed the same characteristics as the wild type. First we characterized the prostaglandin profile of *C. albicans* and *C. parapsilosis* with HPLC and it showed that *C. parapsilosis* do produce PGE2, similarly to *C. albicans*, from the supplemented arachidonic acid. Than we purified *C. albicans* and *C. parapsilosis* PGE2 and we examined the immune modulating effect of these purified prostaglandins on human peripheral blood mononuclear cells derived macrophages (PBMC-DM) with the help of qRT-PCR. When the PGE2 production of *C. albicans* SC5314 and *C. parapsilosis* GA1 wild type, *CpOLE2* heterozygous deletion *(ole2 /OLE2)* and homozygous deletion mutant *(ole2 /ole2)* was measured after the treatment of arachidonic acid by Enzyme-linked immunosorbent assay

(ELISA) we found no difference in the PGE2 production of the mutant strains compared to the wild type strain. Although the C. albicans *OLE2* gene proved to be participating in the PGE2 production, these results are intending that the *CpOLE2* gene do not play a role in the *C. parapsilosis* PGE2 biosynthesis. In order to identify genes that play role in the *C. parapsilosis* PGE2 biosynthesis we carried out qRT-PCR on several genes, chosen from literature and by *in silico* work, after treatment of arachidonic acid. The analysis revealed the significant up regulation of the potential multicopper ferro-O2-oxidoreductase (*CpFET3*) gene after the induction of arachidonic acid. Henceforth while we are creating the *CpFET3* homozygous deletion mutant, we are intending to identify further genes that participate in the PGE2 biosynthetic pathway by carrying out a micro-array analysis.

Supervisor: Attila Gácser
E-mail: grozerb@gmail.com

Role of the HLTF in the tumorigenesis

Adrienn Hajdu

Mutagenesis and Carcinogenesis Research Group, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

The helicase-like transcription factor (HLTF) belongs to the SWI/SNF family of chromatin-remodeling factors. Several SWI/SNF genes are disrupted in cancer, suggesting their possible role as tumor suppressors. Similarly, the HLTF gene was found to be inactivated by hypermethylation in a significant number of colon, gastric and uterine tumors, indicating that HLTF silencing may confer a growth advantage and that HLTF could be considered as a tumor suppressor gene.

Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of K-RAS and BRAF is central to the progression of colorectal cancer. According to published data the K-RAS mutation may be present in 35%-45% of patients with colorectal cancer.

The HLTF gene was silenced by hypermethylation in 45% of colon cancers. Recent studies did not examine the connection between HLTF and other tumor suppressor genes and oncogenes.

The K-RAS mutation and HLTF promoter hypermethylation can be detected equal frequency in colon cancers, but the correlation between them is not examined yet.

We examined about 100 colorectal cancer samples. We analyzed the K-RAS status, the microsatellite status and HLTF promoter hypermethylation. Our results indicate that there are some correlation between the K-RAS mutation and the HLTF promoter hypermethylation. Additionally there is correlation between HLTF hypermethylation and microsatellite instability.

The DNA Mismatch Repair (MMR) system plays an essential role in maintaining the fidelity of DNA replication by correcting single nucleotide mismatches and insertion/deletion (ID)-loops. Many components of eukaryotic MMR have been identified, the molecular mechanism of MMR has been largely demonstrated. Defects in the mismatch repair system result in a mutator phenotype, manifesting as microsatellite instability (MSI) in DNA of affected cells

Recently it has been published that many DNA repair genes (e.g. RecQ helicases, polymarese κ and HLTF) are possible new players of the MMR. We are developing a new useful research tool to verify the contribution of these potential candidates in the MMR process.

Supervisor: Lajos Haracska
E-mail: hadri@freemail.hu

The Role of *Candida parapsilosis* Secreted Aspartyl Proteinase 1 in Host-Pathogen Interactions

Péter Horváth

EMBO Candida workgroup, University of Szeged, Department of Microbiology, Szeged, Hungary

Yeast of the genus *Candida* remain the most prevalent cause of human mycotic diseases worldwide and range in severity from superficial infections to life-threatening systemic diseases. *Candida parapsilosis* is currently the second most common cause of invasive candidiasis. Adhesion to host surface, cell morphology switching between yeast and filamentous growth, biofilm formation and secretion of hydrolytic enzymes such as lipases, phospholipases and aspartyl proteinases are considered to be the most important features for development of candidial disease. *C. albicans* possesses 10 genes encoding secreted aspartyl proteinases (Saps) where expression of a particular Sap isoenzyme depends on the type, site and stage of infection. In contrast little is known about the exact role of *C. parapsilosis* secreted aspartyl proteinases (Sapps) in the development of virulence in host-pathogen interactions. In *C. parapsilosis SAPP1* and *SAPP2* are the 2 annotated secreted aspartyl proteinase genes. Sapp1 and Sapp2 have been studied at the enzymological level. The production of Sapp1 in inducer medium is